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Chapter

Biosafety Issues of Unintended Horizontal Transfer of Recombinant DNA

Getachew Alamnie and Berhanu Andualem

Abstract

Horizontal or lateral gene transfer is the transfer of genetic material from one organism to another organism associated with the unnatural way of reproduction. This way of genetic exchange happens by the stable transfer of genetic material from donor organisms, followed by heritable incorporation to the genome of the recipient organisms. The first explanation of a horizontal gene transfer (HGT) has been a major advance in molecular biology and genetics. Microorganisms like bacteria can acquire genetic material from a variety of sources. The most common DNA transfer mechanisms between microorganisms are conjugation, transformation, and transduction, but there are also other diverse mechanisms of genetic material exchange occurring in nature. Genetically modified organisms (GMOs) often contain recombined genes acquired from different species to enable the expression of new traits in even unlikely or unrelated species. For this, the unintended HGT of rDNA to both prokaryotes and eukaryotes could raise biosafety issues to both human and animal health and the environment. From the current scientific perspective and evidence, the unintended HGT from GMOs to other organisms presents negligible risks to human health and safety or the environment due to the rarity of such events relative to those HGT events that occur in nature.

Keywords: antibiotic resistance, genetic exchange, genetically modified organisms, genomics, potential risks

1. Introduction

In natural way, DNA is usually transferred from parents to offspring over generations following the normal reproduction pathway of the organism involved, and this type of process is called vertical gene transfer; however, DNA can also more infrequently transfer to unrelated species through a process called horizontal gene transfer (HGT) or lateral gene transfer (LGT) [1]. For this, HGT refers to the stable transfer of genetic material from one organism to the other unrelated organism independent of natural reproduction [2–4]. This way of genetic exchange takes place mostly among prokaryotes. But, with very rare exceptions, HGT occurs much less frequently in eukaryotes. This way of genetic exchange between organisms plays a key role in the evolution of genomes and has been recognized within and between the archaea, bacteria, and eukarya domains as well as viruses [2, 3].

HGT enables the exchange of genetic material even between distant species mediated usually by transduction, transformation, or conjugation. This process can be mediated by the integration of viruses (bacteriophages), transposable elements, or integrative plasmids, often via nonhomologous recombination [4].

More recently, concerns have been raised that HGT from genetically modified organisms (GMOs) have adverse effects [1]. Unintended HGT of an introduced gene in a GMO may confer a new trait in another related or unrelated organism, which could be a source of potential biosafety risk to the health of people and animals or the ecosystem. The studies of Xue et al. [5] and Soucy et al. [4] indicated that rDNA process may become an issue of public health concern due to the existence and spread of antibiotic resistant genes (ARGs) among pathogenic bacteria, and even the nonpathogenic becomes pathogenic due to the acquisition of resistant and virulent genes laterally and more recently with the commercial production of genetically modified crops. Similarly, the study conducted by Lu et al. [6] and Zhang et al. [7] discussed of HGT leading to acquisition or modification of traits as antibiotic resistance, virulence, and nitrogen fixation as a means of new characteristics for the new gene acquired organisms. So the objective of this review is to explore the current understanding about unintended horizontal transfer of recombinant DNA in eukaryotes and prokaryotes and mainly the biosafety risks (human and animal health and environmental aspects) associated with unintentional rDNA introduction into the undesired organisms.

2. HGT and the nature of heredity

The first description of a HGT has been a major advance in advanced molecular biology and can even be seen as its founding experiment [4]. A pioneering experiment for the nature of genetic material by Griffith [8], who patented that nonvirulent *pneumococcus* bacterial strain becomes pathogenic simply by contact with virulent bacteria, even bacteria destroyed by heat, capable of modifying heredity by the process called transformation, revealed that the transforming principle can be even transferred from dead virulent bacteria to living nonvirulent bacteria. Following the interesting breakthrough of the transformation principle by Griffith, Avery et al. [9] demonstrated that the remarkable ability of *pneumococci* is to acquire DNA horizontally and suggested that the transforming principle is DNA which is the hereditary material of most living cells, and then Hershey and Chase [10] explored that bacteriophage T2 injects its genetic material (DNA) but not its proteins upon the infection of host bacteria. Also, Watson and Crick [11] proposed that in the double-helical structure of DNA molecule, the genetic information would be contained in the specific sequences of nucleotides.

2.1 Mechanisms of HGT

Exchange of genetic material between bacterial species is mediated by the basic processes of conjugation, transduction, and transformation. The discovery of the various mechanisms of genetic exchange processes by which bacterial genomes recombine and the historical methods are interesting in themselves but also serve to introduce the diverse processes of recombination. In addition to the above well-recognized exchange mechanisms, there are other mechanisms also involved in genetic exchange mechanisms, and the extraordinary capacity of *Agrobacterium* in plants to transfer its genetic material to host cells makes it evolve from phytopathogen to a powerful transgenic vector [12].

2.1.1 Transformation

Transformation is the mechanism of genetic exchange in which free DNA is derived from dead bacteria and is taken up into the cytoplasm of other bacteria. The transformation mechanism of prokaryotes involves integration of free extracellular DNA that becomes incorporated into the genome [13]. Prokaryotic genomes are highly dynamic; they are usually replete with HGT, undergoing continuous gains, often from outside the species, genus, or family, and losses through mutation (i.e., deletion) [14].

2.1.2 Conjugation

Conjugation is a one way transmission mechanism of DNA from one bacterium to another via a "sexual pilus" by which DNA is transported. There are interspecies and intergenus transfers of bacterial DNA by conjugation in food/feed and in the intestine. The transfer of genetic material in *E. coli* conjugation is not reciprocal and one cell, the donor, transfers part of its genome to the other cell, which acts as the recipient by the intermediate of mobile genetic elements, known as integrative and conjugative elements or conjugative transposons, and has been found in many bacterial genomes. Some reports have shown that conjugative transfer in food could have relevant safety consequences due to the mobilization of antibiotic resistance and toxin genes [15].

2.1.3 Transduction

Transduction is the bacteriophage-mediated transfer of host DNA between bacterial cells and occurs when newly forming phages acquire host genes and transfer them to other bacterial cells [16]. Occasionally, the newly forming phage particles mistakenly incorporate a piece of the bacterial genetic material into a phage head in place of phage DNA. This event is the origin of the transducing phage and the phage carrying bacterial DNA can infect another cell. That bacterial DNA can then be incorporated into the recipient cells chromosome by recombination. Studies of model phage host systems have revealed mechanisms of transduction and have led to the development of sophisticated genetic methods [17].

2.2 Detecting patterns of HGT

Microbes acquire DNA from a variety of sources. The development of genome sequencing has revealed that HGT has been a major evolutionary force that has constantly reshaped genomes throughout evolution [4].

3. Introduction to biosafety aspects of recombinant DNA

Theoretically, all genes including highly conserved genes such as ribosomal genes appear to be capable of HGT [18]. Environmental situations including soil, freshwater, seawater, wastes from industry and animals, plant surfaces, human and animal intestines and saliva, and food products may aid HGT. A study investigated by Xue et al. [5] indicated that the transfer of rDNA from GMOs such as transgenic plants to phytosphere bacteria may occur and thus contribute to the undesirable spread of antibiotic resistance determinants and leads to public and animal health risk.

GMOs often contain recombined genes acquired from different species to enable the expression of novel traits in the recipient organism, which leads to new feature and characteristics [7]. As outlined in Akbari et al. [19], some commonly occurring

new characteristics of rDNA in GMOs can make their transgenes more likely to be taken up and expressed in unintended host cell recipients, which poses biosafety issues of the ecosystem.

Nowadays, globally consumers are concerned about the health and environmental consequences of GM foods. Attitudes toward GM foods and willingness to consume these products vary across countries and among consumer groups based on demographic, social, economic, psychological, or religious factors. While the public acceptance of transgenic foods in Japan and the European Union is low, the use of these products in the United States and in some developing countries is fairly high [20]. For instance, Prati et al. [21] found that Italian consumers have a weak intention to consume GM foods. However, Zhu et al. [22] showed that Chinese consumers are willing to consume GM foods and such willingness is affected by their knowledge of the product and risk perceptions.

3.1 Principles and methods of biosafety in HGT

Biosafety means the need to protect human and animal health and environment from the possible adverse effects of the procedures and products of modern biotechnology. For these, there are efforts to protect the environment, human and animal health from the possible adverse effects of the GMOs and products. Because the natures of risks are largely identical for GMOs and non-GMOs, containment measures to handle these risks are largely identical for both. Biosafety pronounces the procedures and policies to be adopted to ensure the environmental and personal safety [23].

3.1.1 Safety and risk assessment of HGT

The risk analysis of genetic engineering reveals the nature and magnitude of risk involved, which in turn helps to decide the levels of biosafety practices. Each form of HGT involves different risk considerations; for genetic engineering, these risks are commonly addressed through legislation [18]. Evaluating threats of HGT of rDNA from GM crops involves estimating both the likelihood of transfer of DNA from GM crops to microorganisms or human cells and the impact of such a transfer event [18].

4. The potential impact of rDNA

The large-scale production of GM crops will necessarily lead to the release and persistence of rDNA in the natural environments [1]. For this, DNA is continually released from living organisms and therefore is not specific to GMOs and the effect thereof should be seen for other organisms present in the same natural system [24]. Foreign DNA from donor organism can take up and integrate into the genome of a recipient organism and will have a deleterious effect due to its interference with the host cell biology and genome structure. HGT processes resemble mutational processes which may occur by chance and repeatedly over time, but a very low proportion of the HGT events will confer a benefit and be retained in the host over time [25]. As a result, unintended HGT of rDNA to both prokaryotes and eukaryotes could pose risks to the environment and human and animal health and these are the concerns of unintended horizontal transfer of rDNA [26].

4.1 Risks due to HGT

The science of rDNA has many potential applications in agriculture, health, environment, and industry. With these different applications, unintended HGT

has become a greater public concern and needs an amendment of procedures and practices regarding its scrutiny and regulation. In the history of organisms' evolvement, their genetic materials have a history of HGT and evolution. Based on the insight from this concept, a study by Soucy et al. [4], Xue et al. [5], Lu et al. [6], and Zhang et al. [7] discussed that the undesired genetic exchange between different organisms could confer novel traits to the recipient organisms, which may result in negative, neutral, or positive effects to the human and animal health and environment.

4.1.1 Gene flow

If there are different allele frequencies in the different populations of a species, then when individuals move to a new population, they will change the allele frequencies in the new population. Environmental implications of gene flow are variable but some of the effects of gene flow are development of superweeds, evolution of new viral pathogens, instability of transgenes in the environment, and creation of pests and pathogens having resistance to new compounds [27]. Concomitantly, secondary effects of gene flow also need to be addressed, including effects on nontarget species, biodiversity disturbance, species displacement and extinction, and disturbance in soil microenvironment and species of ecological concern [28].

4.1.2 Antibiotic resistance

The spread of antibiotic resistant bacteria (ARB) and ARG have become an emerging threat to the global health, which has been extensively found in various environments [1, 24]. By this, HGT which is regarded as one of the major pathways leads to the public health concern by exchanging genetic material that is expressed in the recipient organism [1]. The spread of bacterial antibiotic resistance mainly depends on gene transfer and spontaneous mutation. Mutagenic factors are thought to be the main reason for the induction of antibiotic resistance in the environment [29].

As an alternative, they get mobile genetic factors from other bacteria to obtain antibiotic resistance, which is called HGT. Due to the intensive application of antibiotics in different areas, abundant ARBs and ARGs are frequently detected in environments such as water, soil, and even air, thus posing a potentially health concern [30]. Antibiotics have received special attention due to their potential to promote and maintain bacterial resistance. Particularly, human pathogenic bacteria acquiring ARGs can have severe impacts on human health [31].

4.1.3 Superweediness

Recent studies support that domesticated crops could escape from cultivation (ferality) and become a potential weed. Traits like fast growth rate, self-compatibility (crop traits) could favor weediness [32]. A gain of herbicide-resistance gene through hybridization with GM plants could lead toward its persistence in the agricultural habitat [33]. There exists a contradiction among scientists about the establishment of transgene recipients as weeds in the environment. Based on the risk of increased fitness, certain traits are strong candidates which can enhance chances of competitiveness, such as tolerance to herbicides, resistance to various stress, pathogens and pests, and traits responsible for enhanced growth [34]. Plants can develop several herbicide-resistance mechanisms, such as herbicide detoxification, changes in the intracellular compartmentation of herbicides, target site insensitivity, reduced herbicide entry, reduced herbicide translocation, and target site overproduction [33].

4.1.4 Gene escape and biodiversity loss

Genes can pass on to other members of the same species and possibly other species at the gene, cell, organism, and ecosystem levels. Although research is inconclusive, there is scientific consensus that once widely released, recalling transgenes. The widespread commercial production of GM crops, especially herbicide tolerant crops, poses serious threats to the ecosystem complexity and reduction in biodiversity. To a broader sense, it could be concluded that biodiversity is negatively affected by the cultivation of GM crops [35].

4.1.5 Evolution of herbicide-resistance weeds

In the large field of agriculture, there are many evolutionary events that could simultaneously interplay for the emergence of herbicide-resistance gene [36]. In tolerance development, various mechanisms could help the plant, such as target site overproduction, modification in intracellular herbicide compartmentation, minimal herbicide absorbance and translocation, herbicide detoxification, and insensitivity to target site [37].

4.1.6 Resistance to insecticide and pesticide

Controlling pests through conventional and chemical techniques have been proven to be challenging, as evolution of insecticide and pesticide resistance has been witnessed in many cases. More specifically, the possibility of evolution of Bt-resistant insect pests cannot be negated because of the constitutive expression of Bt toxins in all plant tissues that imparts higher selection pressure on target species. Use of Bt biopesticides by organic farmers lead to resistant diamondback moth populations in Central America, Florida, Japan, Philippines, Hawai, and China [38].

5. HGT of rDNA to eukaryotes

The uptake of food-derived DNA into host cells or tissues has been raised as a potential concern that is related to the introduction of GMO-based food sources [39, 40]. Among the concerns regarding GM foods are the possible consequences of HGT of recombinant dietary DNA to animal cells [40]. The exposure of the gastrointestinal tract (GIT) to dietary DNA is related to the extent of food processing, food composition, and the level of intake. A minor amount of fragmented dietary DNA may resist in the digestive process of animal feeding [40]. Feed-derived rDNA are taken up from the GIT and detected in the muscle, liver, spleen, and kidneys in chicken and cattle, and it has been estimated that approximately 0.1–1% of dietary DNA is absorbed from the gastrointestinal tract [41].

DNA is continually introduced into the GIT as a natural part of food. Whereas, the majority of feed-derived DNA is broken down during digestion, and several studies have now shown that minor proportions of feed-derived DNA survive immediate degradation and reach the bloodstream in various animals [42] or are detectable as minor fragments in feces. The fate of chromosomal DNA in the GITs of humans and animals has recently received increased attention due to the introduction of novel ingredients derived from GMOs in the food chain. Biological risk assessment of GMOs has exposed knowledge gaps related to how DNA is degraded or survives degradation in various compartments of the GIT.

5.1 The GIT of human

The human body is a complex ecosystem harboring trillions of bacteria in different body habitats, and the GIT alone is inhabited by 10^{13}–10^{14} bacteria [43]. Dysbiosis of the gut microbiota is concerned in a wide range of diseases such as inflammatory bowel disease, diabetes, and cardiovascular disease [44]. The dynamics of these bacterial communities is complex. However, one hallmark of these communities is that bacteria can share different phenotypic traits through a transfer of genetic material.

5.2 DNA in food

The broad application of sensitive polymerase chain reaction (PCR) technology has thus exemplified the widespread occurrence and persistence of DNA molecules in various food sources, including processed food such as corn chips and chocolate [45]. Thus, the overall concentration and distribution of DNA of a size that enables entire protein-coding genes to be horizontally acquired from various food sources by host cells or bacteria remains largely undetermined. Studies conducted by Duggan et al. [46] have demonstrated that the persistence of DNA in food revealed that processing often decreases the size of DNA, and such molecules can be undetectable in extensively processed food.

5.2.1 DNA stability in the digestive tract

Most free digested DNA molecules entering the digestive system undergo substantial degradation by enzymes attacking DNA, which are released from the pancreas and by the bacteria present in the intestine [47]. DNA fragments are excreted in the feces with variation in the degradation efficiency between mammals. For this, Chowdhury et al. [48] reported that maize DNA could be detected in pig feces. Most studies on DNA stability in the digestive systems of mammals have used purified DNA and may therefore not capture the impact of various food components, treatments, and locations on DNA degradation and stability. Although deoxyribonuclease I (DNase I) is detected in saliva, it is believed that DNA digestion starts in the stomach.

6. HGT of recombinant DNA to prokaryotic cells

HGT of transgenes into pathogenic beneficial or environmental microorganisms resulting in potential unanticipated fitness effects has been voiced as a potential biosafety issue. Microorganisms are responsible for the majority of organic matter decomposition; thus, microorganisms present in the human GIT and in agricultural environments experience continual exposure to DNA released from themselves and the organisms in their immediate surroundings [49]. However, in rare circumstances, foreign DNA may also be integrated into the bacterial genome. Experimental studies do not suggest bacteria integrating into foreign unrelated chromosomal DNA at measurable frequencies over the limited time span (hours to days) [50]. Thus, microbial communities are in some cases already exposed to naturally occurring counterparts to these protein encoding genes [50, 51].

7. Concluding remarks

From this scientific review, HGT from GMOs to other organisms presents negligible risks to human and animal health and safety or the environment due to

the infrequency of such events relative to those HGT events that occur in nature. Ku and Martin [52] have indicated that eukaryotes do not acquire genes through continual HGT like prokaryotes. The risk assessment of a transgenic bacterium must consider the potential for transfer of introduced genes to other microorganisms in the environment. The biosafety aspects of unintended horizontal gene transfer from GM crops that are currently commercialized may become a potential health concern.

Abbreviations

ARB	antibiotic resistant bacteria
ARGs	antibiotic resistant genes
GIT	gastrointestinal tract
GMOs	genetically modified organisms
HGT	horizontal gene transfer
PCR	polymerase chain reaction
rDNA	recombinant DNA

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